8 GAS CHROMATOGRAPHY

8.1 Objectives

8.1.1 Understand the theory of gas chromatography (GC).

8.1.2 Become familiar with the practical aspects of GC.

8.1.3 Study the components of a gas chromatograph, and understand their function and specifics of operation.

8.1.4 Become proficient in the operation of the various GCs used in the toxicology section.

8.1.5 Perform qualitative and quantitative GC analyses of extracts from biological specimens for the presence of chemicals.

8.1.6 Examine and interpret chromatographic printouts.

8.1.7 Understand the use of internal and external standards, and quality control as applied to GC.

8.2 Estimated Time: Two months

8.3 Methods of Instruction

8.3.1 Lectures

8.3.1.1 Principles of gas chromatography

8.3.1.2 Parameters affecting the separation process and resolution of peaks

8.3.1.3 Components and operation of GC

8.3.1.4 Types of injectors and injection techniques

8.3.1.5 Types of columns

8.3.1.6 Types of detectors

8.3.1.7 GC optimization

8.3.1.8 Result interpretation

8.3.2 Literature Review

8.3.2.1 *Capillary Chromatography Training Manual*, Restek Corporation.

8.3.2.2 *HP6890 Series GC Operating Manual, 1. General Information*, Hewlett-Packard.

8.3.2.3 *HP6890 Series GC Operating Manual, 2. Inlets*, Hewlett-Packard.

8.3.2.4 Hyver, KJ. et al, *High Resolution Gas Chromatography*, 3rd Ed. 1989, Hewlett-Packard Co.

8.3.2.5 M Moffat, A.C., editor. *Clarke's Analysis of Drugs and Poisons*, 3rd edition. London: The Pharmaceutical Press, 2004 pp 425-499.

8.3.2.6 Rood, D. *A Practical Guide to the Care, Maintenance, and Troubleshooting of Capillary Gas Chromatography Systems*, 3rd Revised Ed. 1999, Wiley-VCH.

8.3.2.7 Toxicology Technical Procedures Manual.

8.3.2.8 Willard, H.H., Merritt, L.L. Jr., Dean , J., Settle, F.A., *Instrumental Methods of Analysis*, 7th Ed. 1988, Wadsworth Pub Co., pp 540-578.

8.3.3 Demonstration

8.3.3.1 Use of gas chromatographs will be observed from beginning to end and notes will be taken by the Trainee.

8.3.4 Laboratory Exercises

8.3.4.1 Determine the retention time and relative retention time (using the GC/NPD and methapyrilene as the internal standard) of the following drugs: amitriptyline, caffeine, cocaine, desipramine, dextromethorphan, diazepam, diphenhydramine, doxepin, doxylamine, fluoxetine, imipramine, ketamine, lidocaine, meperidine, methamphetamine, methadone, nordiazepam, nortriptyline, norpropoxyphene, phencyclidine, promethazine, propoxyphene, quinine, sertraline, trazadone and zolpidem.

8.3.4.2 Run barbiturate extracts from Section 7 (Extraction and Derivatization) on the GC/NPD. Identify the peaks.

8.3.4.3 Run 10 previously analyzed cocaine extracts from Section 7 (Extraction and Derivatization) on GCMS. Use the Chemstation software to quantitate cocaine, cocaethylene and benzoylecgonine (Section 10 Quantitation).

8.4 Evaluation

8.4.1 Written Examination

8.4.1.1 This will be administered as a “take home” exam.

8.4.2 Laboratory Competency Testing

8.4.2.1 Prepare the GC/NPD for analysis of 10 base extracts. Run the 10 unknown base extracts from Section 7 (Extraction Module) on the GC/NPD. Identify all drugs by retention time.

8.4.3 Courtroom Exercise

8.4.3.1 The Trainee must be capable of answering questions on this Module such as would be expected in a courtroom scenario.

8.5 Examination Questions

8.5.1 What is gas chromatography?

8.5.2 What types of information are obtained from GC?

8.5.3 Draw a schematic diagram of a gas chromatograph and describe the function of each component.

8.5.4 Describe the different types of stationary phases used in the Toxicology Section.

8.5.5 List three different modes of sample introduction and state the advantages and disadvantages of each.

8.5.6 What factors govern the amount of sample to be injected? How much sample can the average capillary column hold? What factors influence this?

8.5.7 What temperature should the injection port be under normal circumstances and why?

8.5.8 What type of septum is recommended for GC work and why?

8.5.9 What is an injection port liner? What is it made of? Why is it used? Describe the packing process including the materials used.

8.5.10 What is a split ratio? How is it calculated?

8.5.11 Describe packed, capillary and megabore GC columns and state applications and limitations of each.

8.5.12 Describe the various GC detectors used in the toxicology section (i.e. FID, NPD, ECD) stating the application and limitation of each.

8.5.13 Describe the advantages and disadvantages of isothermal vs temperature programming.

8.5.14 Why is it necessary to regulate the carrier gas flow?

8.5.14.1 How is this done?

8.5.14.2 What factors influence the optimum flow rate for a given carrier gas?

8.5.14.3 If the carrier gas is too fast or too slow, how will it affect the peak shapes?

8.5.14.4 How will it affect the detector?

8.5.15 What is “make-up” gas? How and why is it used?

8.5.16 Explain the following statement: *response is proportional to the number of carbon atoms in the sample.* What type(s) of detector is this statement applicable to?

8.5.17 Discuss the operation of an autosampler.

8.5.18 What are the possible causes and remedies for the following GC problems?

8.5.18.1 No peaks

8.5.18.2 Tailing peaks

8.5.18.3 Leading peaks

8.5.18.4 Split peaks

8.5.18.5 Baseline drift

8.5.19 What is column bleed?

8.5.20 When and why are columns conditioned? Describe the process.

8.5.21 Define the following terms:

Carrier gas
Height equivalent theoretical plate
Mobile phase
Resolution
Stationary phase
Kovat's retention index
Partition coefficient
Retention time
Theoretical plates
Column efficiency
Make-up gas
Van Deemter plot
Phase ratio
Selectivity
Trennzahl number
Flow rate
McReynold's constant
Relative retention time
Signal to noise ratio

♦ **End**